

## MAINTENANCE OF CONSTANT LEAF TEMPERATURE BY PLANTS—II. EXPERIMENTAL OBSERVATIONS IN COTTON

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UPCHURCH D. R. and MAHAN J. R. *Maintenance of constant leaf temperature by plants—II. Experimental observations in cotton*. ENVIRONMENTAL AND EXPERIMENTAL BOTANY 28, 359–366, 1988.—Plants are generally assumed to be eurythermal poikilotherms. Several species have, however, exhibited narrow temperature ranges for optimum enzyme function that are uncharacteristic of eurythermic organisms. In order to determine the extent to which cotton plants are eurytherms, the leaf temperatures of cotton (*Gossypium hirsutum* L.) grown in a fiberglass-covered greenhouse were monitored under eurythermal conditions. Leaf temperature, relative humidity, global and photosynthetically active radiation, air temperature and water use were measured continuously for 60 days. Homeothermic behavior by cotton plants was consistently observed when three environmental conditions were satisfied. These conditions were: (1) sufficient energy input to raise the leaf temperature to 27°C, (2) sufficient water available for transpiration, and (3) humidity low enough to allow evaporative cooling. Even with wide variations in air temperature (27–40°C), cotton maintained a normative plant temperature ( $T_n$ ) of  $27 \pm 2^\circ\text{C}$ . On the basis of this observation we conclude that cotton plants can function as limited homeotherms.

### INTRODUCTION

INVESTIGATIONS into the relationship between the temperature of the plant and that of its environment have suggested that plants are poikilotherms.<sup>(7,11)</sup> Poikilothermy requires that the temperature of the organism be controlled by its environment. Hence, in a constant temperature environment a poikilotherm will be homeothermic and in a variable temperature environment a poikilotherm will be eurythermic. Given that most plants live in environments that are subject to wide variations in temperature (i.e. eurythermal) it has been assumed that plants are eurythermal poikilotherms.

The temperature of a plant canopy is determined by the balance between energy gain and energy loss. Evaporative cooling of transpiring leaf surfaces often causes the canopy temperature to be less than the ambient air temperature. The

resulting differential between canopy and air temperature has been used in estimating the rate of transpiration.<sup>(5)</sup> The temperature of a crop canopy relative to air temperature has also been used as a measure of crop water stress.<sup>(6,13)</sup> The relationship described by JACKSON *et al.*<sup>(6)</sup> indicates that the temperature differential between well watered plants and the ambient air is affected by the gradient in water vapor pressure between the leaf and the atmosphere at a particular temperature, and therefore leaf temperature is not constant. However, GATES<sup>(4)</sup> noted thermal homeostasis of plant leaves in the field, stating “. . . it was a great surprise to find such homeostasis among plant leaves in the field.”

Differences in thermal dependence between enzymes from eurythermal and homeothermal organisms have demonstrated that the enzymes from an organism are adapted to function opti-

mally over the thermal range that is normative for that organism. Thus the enzymes from eurytherms function optimally over a wide range of temperatures while enzymes from homeotherms function optimally over thermal ranges that are characteristically narrow.<sup>(12)</sup> The thermal dependencies of plant enzymes that we have observed are uncharacteristically narrow for enzymes from eurythermal organisms.<sup>(2,9)</sup>

MAHAN and UPCHURCH,<sup>(10)</sup> in a companion paper, have proposed that plants may control their temperature within relatively narrow and species specific ranges. The objective of this project was to evaluate the hypothesis of limited homeothermy<sup>(10)</sup> for cotton plants by monitoring their leaf temperatures relative to vapor pressure deficit, air temperature, and global radiation. These factors are expected to impact the energy balance and thereby, the temperature of the leaf surfaces of the plant canopy.

## MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L. cv. "Paymaster 404") was grown in a fiberglass-covered greenhouse (9 × 9 m) in containers with a soil volume of 18 l. Soil (Acuff, fine-loamy, mixed, thermic, Aridic Paleustoll) was air dried, ground, and sieved to pass a 2-mm opening before being packed into pots to a density of 1.44 Mg/m<sup>3</sup>. Care was taken to insure identical soil volumes for each container. The surface of all pots was covered with an opaque plastic film barrier to suppress evaporation of water from the soil surface, and the pots were sealed at the bottom to prevent drainage. A small opening was provided in the barrier, by sealing a styrofoam cylinder (50 mm diameter) into the plastic, through which the plant could grow. This cylinder extended from the soil surface to approximately 10 mm above the plastic. There was an air gap between the plastic and the soil surface of approximately 15 mm. In previous experiments this procedure resulted in less than 0.1 kg of water loss through surface evaporation over a 60-day period. Based on soil water retention-curves determined in our

laboratory, sufficient water was added to bring the water content to field capacity (0.26 m<sup>3</sup>/m<sup>3</sup>) 4 days prior to planting. Three seeds were planted in each pot on 27 April 1987, and plants were thinned to one plant per pot after emergence on 4 May 1987. A total of 60 pots was located in the greenhouse, with four pots randomly chosen for continuous, detailed measurements. The pots were arranged in rows on benches in the greenhouse such that a canopy similar to the field situation was developed as the plants grew. There was approximately 2.5 l of water available for plant uptake from each pot. Available water was estimated as the difference between the water content at field capacity (0.26 m<sup>3</sup>/m<sup>3</sup>) and that at 1.5 MPa water potential (0.12 m<sup>3</sup>/m<sup>3</sup>). When approximately 1.5 l had been extracted, based on pot mass measurements, nutrient solution was added to each pot. The amount added was the average of that lost by the four pots weighed continuously. The nutrient solution consisted of 0.3 g of Miracle Gro\* (15% N, 30% P<sub>2</sub>O<sub>5</sub>, 15% K<sub>2</sub>O) per liter of water.

The air temperature inside the greenhouse was controlled with cross ventilation and evaporative coolers for high temperatures and gas-fired overhead heaters for low temperatures. Side ventilation panels were automatically opened or closed at preset temperatures as the primary temperature control. At a slightly higher air temperature cross ventilation was assisted with a fan. Evaporative cooling was added at a third preset temperature. If the air temperature dropped below the preset temperature the side ventilation panels were automatically closed. If the temperature continued to decline, the overhead heaters were actuated. This system adequately controlled the lower temperature with the heaters but the cooling system was inadequate to maintain the desired temperature.

Plant temperatures, pot mass and environmental parameters were monitored continuously during the experiment. All measurements were collected with an electronic data acquisition system (Campbell Scientific, Model CR-7 Data Logger). Input channels were scanned at 15 sec inter-

\* Mention of a specific trademark does not imply endorsement by USDA to the exclusion of other appropriate equipment.

vals and averages or totals were calculated and stored every 15 min.

Global radiation was measured with a Licor Model LI-200SCZ pyranometer and photosynthetically active radiation (PAR) with a Licor Model LI-190S quantum sensor. These sensors were mounted approximately 2 m above the benches holding the pots. Because the experiment was conducted inside a fiberglass greenhouse, the radiation sensors were sometimes shaded by solid structural members. Air temperature and relative humidity (RH) were measured with a Campbell Scientific Model 207 probe mounted under a reflective surface approximately 0.5 m above the plant canopy. Vapor pressure deficit (VPD) was calculated from the RH and air temperature using a subroutine provided in the data logger. Accuracy of the RH and air temperature probe was checked periodically throughout the experiment using a Weathertronics Model 5230 psychrometer. Wind run was also measured 0.5 m above the canopy using an R. M. Young, 3 cup anemometer Model 12102. The anemometer was calibrated against standards prior to the experiment.

The temperatures of several points on the four plants being weighed were measured with 0.3 mm diam. copper-constantan thermocouples constructed from 36 gauge wire. Shortly after emergence, a thermocouple was attached to the underside of one of the cotyledons. This was accomplished by tying the lead wire to the petiole and bending the wire such that the thermocouple bead was in contact with the leaf. Approximately 2 weeks after emergence, a second thermocouple was placed near the apical meristem of the plant. Care was taken to place the bead in contact with the expanding leaves without damaging the tissue. Because these were near the top of the plant, they were exposed to direct solar radiation during portions of the day. When four true leaves were present on the plants, a third thermocouple was placed in contact with the most recently fully expanded leaf using the procedure described for the cotyledons. As new leaves were formed, this thermocouple was moved so that it was always attached to the underside of a leaf near the top of the plant which was fully exposed to incoming radiation. A fourth thermocouple was placed in contact with a fruiting structure on each plant on

9 June. The bead was placed inside the bract such that it was not exposed to solar radiation. As the flower and later the boll developed, the thermocouple remained in contact with the same fruiting structure. When the cotyledons senesced, the thermocouple previously attached to it was moved to another leaf located near the bottom of the plant that did not receive direct solar radiation during any portion of the day. The two leaf positions (top and bottom of canopy) were chosen to establish a large difference in radiant load. The position of all thermocouples was checked at least once each day to insure that they remained in contact with the plant tissue.

The mass of the four pots was measured continuously using electronic load cells (Genisco Model DWB-10) attached to the arm of Model 744 Detecto Bench Scales. Although the load cell output was temperature corrected, the mechanical bench scale was influenced by temperature changes, which limited the resolution of the system. Therefore pot mass measured at midnight, which maintained a fairly stable temperature, was used for determination of total daily water use.

## RESULTS AND DISCUSSION

Frequency, defined as cumulative time at a particular temperature, was calculated for each 1°C increment between 20 and 40°C. Time above 40°C or below 20°C was accumulated into the 41°C or 19°C increments, respectively. The frequency distribution of time at a given temperature is shown in Fig. 1A for air temperature and the temperature of an upper canopy leaf, over a period of 54 days. The leaf temperature with the highest frequency was 27°C while the highest frequency for air temperature occurred at 30°C. The results presented in Fig. 1 are typical of the results for each leaf measured in this project. The normative plant temperature ( $T_n$ ) was defined in the companion paper as the leaf temperature that is preferentially maintained by the plant. This temperature was determined from a plot of the difference between the frequency of leaf temperature and the frequency of air temperature (Fig. 1B). Since plant leaves are cooled by transpiration the frequency of high leaf temperatures (those greater than  $T_n$ ) must be less than that for

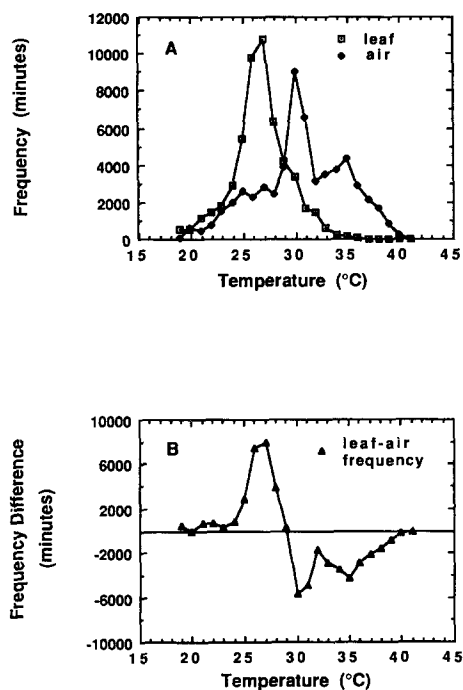


FIG. 1. (A) Frequency, cumulative time at temperature, for upper canopy leaf and air temperature. (B) The difference between leaf temperature frequency and air temperature frequency. The curve in panel B is the difference between the curves in panel A.

the same air temperature. The total time must be equal for leaf and air, therefore, the frequency of leaf temperatures near  $T_n$  should be greater than that for the same air temperature. A maximum in Fig. 1B occurs at 27°C; this is the temperature at which the largest positive deviation between the frequencies for leaf and air temperature occurs, that is, the preferentially maintained temperature. Although in this experiment the highest frequency of leaf temperature also occurred at  $T_n$  (Fig. 1A), it will not be true in all environments. For example, in an environment in which the maximum frequency for air temperature occurred at a temperature below  $T_n$ , the maximum deviation and maximum frequency for leaf temperature will not occur at the same temperature. However, the temperature of maximum deviation would again indicate the  $T_n$ . Although we have defined  $T_n$  to be a particular temperature, 27°C, we can only be positive that it is in

the range between 25° and 29°C. We will therefore refer to  $T_n$  for cotton as 27°C  $\pm$  2°C, throughout this paper.

Mean air temperature vs mean leaf temperature (upper and lower canopy) over the course of the experiment is shown in Fig. 2. Each point represents the hourly average of the leaf temperatures of the four plants monitored in the experiment and the hourly average air temperature. A linear relation between air and leaf temperature was found for air temperatures below 27°C. Plant temperature was approximately 1°C below air temperature over this range. Radiant cooling of the leaf surface below air temperature may be responsible for this difference, though the consistency of the differential argues against such an interpretation. Also, since the experiment was conducted inside a greenhouse, net radiation at night would be close to zero. A more plausible explanation is a systematic bias in the temperature measurements. Air temperature was measured above the plant, while leaf temperatures were measured at the leaf surface. The difference may have been due to a temperature gradient between the location of the air temperature sensor and the air immediately surrounding the plant. This gradient would be maintained during times of cool air temperatures because the cross ventilation fan would not be operating.

At air temperatures above 27°C the plant was cooler than the surrounding air with measured leaf temperature increasing roughly 1°C for each

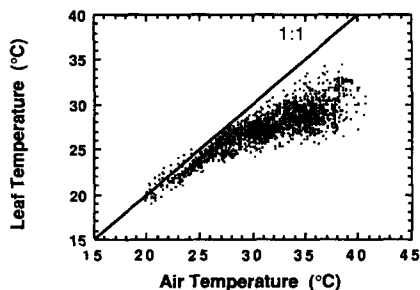


FIG. 2. Leaf temperature vs air temperature from day of year 127 to 180. Each point represents the hourly average of the leaf temperature from four plants and two leaf positions (upper and lower canopy) vs hourly average air temperature.

3°C increase in air temperature. This upward trend in plant temperature does not conform to our hypothesis of limited homeothermy. However, the observed increase in temperature with increasing air temperature is a characteristic of leaf temperature measurements using thermocouples. For example, a thermocouple bead in contact with the underside of a leaf will sense a combination of the leaf temperature and the temperature of the air surrounding the leaf. Therefore, with a constant leaf temperature, the temperature sensed by the thermocouple must increase with increasing air temperature. However, the thermocouple temperature will increase less than the increase in the air temperature. Therefore, in this study any increase in the leaf temperature above 27°C must have been less than that measured by the thermocouple, suggesting that leaf temperature is even more stable than indicated in Fig. 2.

During both day and night, a separation of leaf and air temperature was evident above 27°C (Figs 3A and 3B). The majority of air temperatures below 27°C occurred during night

hours (Fig. 3B) and there is excellent agreement between air and leaf temperature in this region. During the day a similar agreement between leaf and air temperature is apparent at air temperatures below 27°C (Fig. 3A). Above 27°C leaf temperature increased with increasing air temperature in a manner similar to that seen in Fig. 2, for both day and night. However, as discussed previously, the true increase in leaf temperature may be somewhat less than that shown in Fig. 3.

LINACRE<sup>(8)</sup> presented results of simultaneous leaf and air temperature measurements from 42 previously published reports. Regression analysis of these results indicated that leaf temperature rose continuously with increasing air temperature from 8°C to greater than 50°C. Below an air temperature of 35°C, leaf temperature was above air and above 35°C the leaf was cooler than air. The results he presents were collected "about noon at times of bright sunshine", and were from several plant species. LINACRE's results support our observation of leaf cooling at high air temperatures and also provide evidence that leaf temperatures may exceed air temperature under certain conditions. However, his results do not provide insight into homeothermic behavior by plants. His observation of an equivalent point at 35°C is likely a fortuitous result of the data available at the time of his analysis. Rigorous theoretical analysis by CAMPBELL<sup>(3)</sup> suggests that there may be an equivalence point temperature which is a function of leaf shape as well as several environmental parameters.

The apparent discontinuity at 27°C in the results presented in Figs 2 and 3 suggests a switch type mechanism that allowed the cotton plants to avoid high temperatures. A similar result has been observed by ALTHAWADI and GRACE<sup>(1)</sup> in desert cucurbit. In their work transpiration increased from 0.13–0.17 g/m<sup>2</sup> sec to 0.6 g/m<sup>2</sup> sec when the plants were exposed to air temperatures in excess of those occurring naturally. This resulted in a depression of leaf temperature as much as 7° below air temperature. They suggested the presence of a "finely-tuned control mechanism . . . enabling the plant to avoid lethal temperatures".

As discussed in our previous paper<sup>(10)</sup> high humidity will limit the range of evaporative cooling. Evaporative cooling can only lower the temperature of the leaf surface to the point where

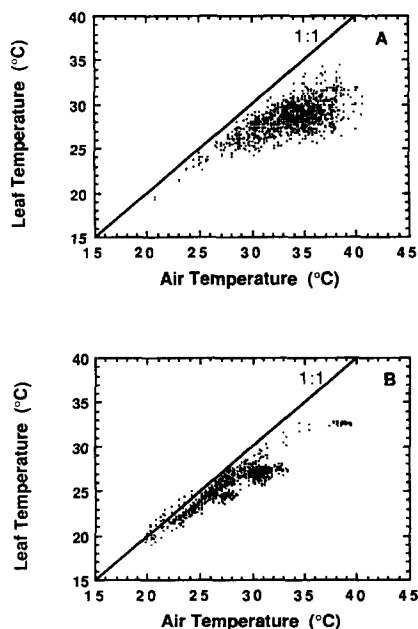


FIG. 3. Leaf temperature vs air temperature from day of year 127 to 180 for: (A) daytime (0800–2000) and (B) night-time (2000–0800).

the air in the boundary layer surrounding the leaf is water saturated. Although measurement of RH in this experiment did not indicate that this limitation occurred it is possible that the small group of points at a leaf temperature of 33°C in Fig. 3B could have resulted from a high humidity surrounding the leaf. Relative humidity was measured above the canopy which may not be representative of conditions at the leaf surface with the calm conditions of the greenhouse. CAMPBELL<sup>(3)</sup> developed a thorough analysis of the energy exchanges of plant leaves and canopies. His theoretical analysis establishes, from physical principles, that vapor concentration within a canopy may be substantially higher than that above the canopy.

CAMPBELL<sup>(3)</sup> also addresses the complexity of the analysis of energy and water vapor exchanges within a canopy. Values of the environmental factors affecting these exchanges near the bottom of a canopy will often be substantially different from those at the top of the canopy. Thus we might expect leaf temperatures to vary within the canopy. The results presented in Figs 2 and 3 are from two positions (upper and lower canopy) and display similar behavior. Under our hypothesis of limited homeothermy we would expect that all leaves would tend toward the same temperature, which is supported by these results.

The overall results of this project represented by Figs 1–3 suggest that the cotton plants in this study maintained leaf temperatures within a rather narrow range when air temperature exceeded 27°C. The plants displayed an apparent normative leaf temperature of  $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for both light and dark periods. In order to help define the mechanism and some environmental constraints involved in the maintenance of  $T_n$ , analysis of the data on a diurnal time scale is useful. Since it is impossible to present all of the results from this project, results from a few representative dates will be discussed in detail. The results presented in the following figures are averages of four plants and are representative of behavior observed throughout the experiment.

For day of year (DOY) 151 and 152 night air temperatures were below  $T_n$  (27°C) for significant periods (Fig. 4A). When the air temperature was below 27°C the leaf temperatures were usually within 1°C of the air temperature

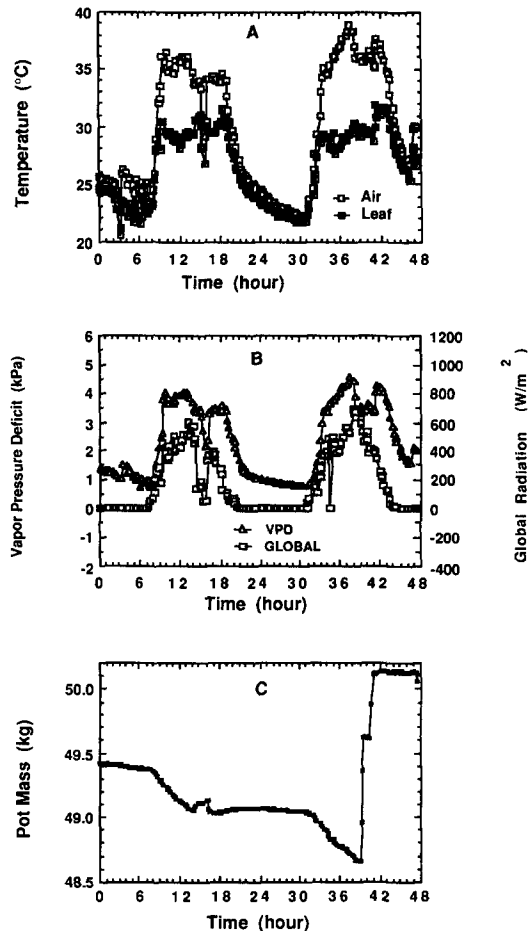


FIG. 4. Diurnal values of: (A) leaf and air temperature, (B) vapor pressure deficit and global radiation, and (C) average pot mass for day of year 151 (0–24 hr) and 152 (24–48 hr).

( $T_a$ ). When the temperature of the leaf reached  $T_n$  (i.e.  $T_a \geq T_n$ ) transpirational cooling effectively maintained the measured leaf temperature within the range of 26–30°C, while  $T_a$  rose as high as 39°C (Fig. 4A). During this period vapor pressure deficit ranged from 0.5 to 4.5 kPa and global radiation from 0 to 670 W/m². Total mass of the pot over this time period is shown in Fig. 4C. The increase in mass between 1500 and 1600 on day 151 was caused by a simultaneous drop in air temperature. This is a result of the temperature sensitivity of the balance and is not a

true weight gain. The plants were irrigated in the afternoon of the second day. The plant transpired approximately 0.5 kg of water during each of the 2 days, with no apparent loss of water when the leaf temperature was below  $T_n$ .

The similarity between leaf and air temperature at night may be a result of the greenhouse environment. Very little net loss of energy by reradiation would be expected from the greenhouse, thus minimizing the possibility of radiational cooling of the leaves. Under clear sky and calm conditions in the field, leaf temperatures substantially below the ambient air temperature can occur.

The period from DOY 155 to 156 represents conditions in which the air temperature was always greater than or equal to the  $T_n$  ranging from 27 to 39°C (Fig. 5). The leaf temperature remained relatively constant over this period (light and dark). Vapor pressure deficit and global radiation ranged from 2 to 5.5 kPa and 0 to 670 W/m<sup>2</sup>, respectively (Fig. 5B). The time course of the pot mass is shown in Fig. 5C. Approximately 0.7 kg of water was transpired during each of the days. Observation of the maintenance of constant leaf temperature several degrees below  $T_a$  during the night strongly suggests that transpirational cooling was occurring. The decrease in mass of the pot over the 8-hr dark period indicates the loss of 0.05 kg of water (Fig. 5C). Incoming solar radiation over this same time period was zero (Fig. 5B), therefore the amount of transpiration required to maintain  $T_n$  was greatly reduced as compared to daylight conditions. The combination of maintenance of the leaf at  $T_n$ , below  $T_a$ , and the loss of water indicates control of leaf temperature in the dark by transpiration.

### CONCLUSIONS

Cotton plants apparently maintain a characteristic temperature of  $27^\circ\text{C} \pm 2^\circ\text{C}$ , which we have defined to be the normative plant temperature.<sup>(10)</sup> Plants maintained their leaves at  $T_n$  over a variety of environmental demands and across a range of developmental stages. In this experiment the primary limitation to homeothermy was low air temperature. At air temperatures above  $27^\circ\text{C}$  the plants maintained their leaf temperature at  $T_n \pm 2^\circ\text{C}$  by transpirational cooling. Other limi-

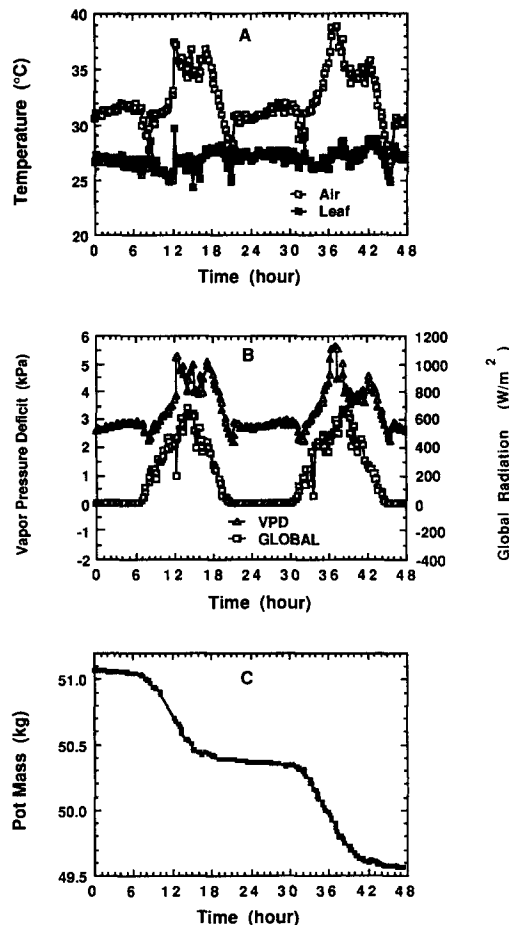


FIG. 5. Diurnal values of: (A) leaf and air temperature, (B) vapor pressure deficit and global radiation, and (C) average pot mass for day of year 155 (0–24 hr) and 156 (24–48 hr).

tations to homeothermy in plants have been discussed in our previous paper<sup>(10)</sup> such as high humidity and lack of water for transpiration. Apparently these limitations did not occur in this experiment.

It has been proposed that the enzymes of cotton are limited to optimal function within a thermal kinetic window of  $23.5\text{--}32^\circ\text{C}$ .<sup>(9)</sup> These results demonstrate that a well watered cotton plant is able to maintain its leaf temperature, under a variety of environmental conditions, well within the limits of this thermal kinetic window.

The proposal that plants are limited homeo-

therms<sup>(10)</sup> has implications that touch diverse areas of plant studies. The interaction between the plant and its environment will perhaps require closer scrutiny in light of the ability of the plant to maintain  $T_n$  with great precision. The apparent ability of the cotton plant to remain homeothermal in a eurythermal environment suggests that the role of water use by the plant may need to be re-evaluated in several respects. It has often been suggested that the use of water by the plant is basically a passive process and simply a consequence of the plant having its roots in a wet soil and its leaves in a dry atmosphere (i.e. the plant is a wick). In light of the homeothermic behavior seen in this study it is apparent that water use by the plant is an active process through which the plant is able to modify its temperature in such a way as to limit thermal variation.

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